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A DEAE CELLULOSE BATCH TECHNIQUE FOR ANTILYMPHOCYTE GLOBULIN PREPARATION

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IN THIS STUDY an attempt was made to produce a less toxic heterologous antilymphocyte globulin (ALG) by first improving the absorption of the raw serum (ALS) and then by attempting to remove from it only the gamma G globulin (ALGG) with a diethylaminoethanol (DEAE) cellulose batch technique. The serum was obtained from horses which had been immunized with dog or human splenic lymphocytes (1).

RESULTS

Absorption: With the original technique (1) the horse serum was decomplemented by heating to 56° C. and then absorbed against red cells and pooled serum of the species to be treated; decomplementation was done first to avoid hemolysis of the added red cells. The procedure had two flaws. First, the use of serum (rather than plasma) for absorption precluded the removal of antifibrinogen and antithrombocyte antibodies. Secondly, the removal of horse antibodies against other human plasma proteins was made inefficient by the absence of complement at this stage. These objections were eliminated or minimized. Whole blood was collected from multiple donors using ethylene diamine tetra-acetic acid (EDTA) disodium as an anticoagulant in order to prevent platelet aggregation. The red cells, plasma, and platelets were separated with differential centrifugation. The human (or dog) plasma was added to the unaltered horse serum and incubated for 2 hr. at 37° C. and 18 hr. at 4° C. With the addition of one part human or dog plasma to 10 parts of horse serum, most of the horse precipitating antibodies were eliminated. Before this incubation, the platelets isolated earlier were added to the mixture in quantities determined by the premeasured titers of the antiplatelet activity in the ALS and by standard curves showing the number of platelets needed at various titer levels. Platelet absorption did not reduce the antiwhite cell titer. The supernatant was now decomplemented by heating at 56° C. for 30 min. and absorbed against red cells; hemolysis did not occur. The final product had no hemoly-

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ins, very low titers of hemagglutinins, few antiplatelet antibodies, and antihuman protein antibodies only occasionally in the a_2 region (with immunoelectrophoresis).

Globulin separation: The conditions of pH and osmolarity were defined whereby ALGG could be removed by adding DEAE cellulose to the absorbed horse serum (2). However, the quantities of DEAE were too large to permit application of the method for mass production. Consequently, a preliminary step was introduced of precipitation of the absorbed raw globulin with 0.4 saturated ammonium sulphate. The precipitate was then dissolved and batch mixed with much smaller quantities of DEAE cellulose at a pH of 7.0 (adjusted with phosphate buffer) and an osmolarity of 0.01. The protein removed from the supernatant after mixing was pure ALGG in 7 of 12 batches of antihuman material. Variable quantities (usually traces) of beta globulin contaminated the other five; the protein concentration was 1.9 to 3.6 gm%, the leuko-agglutination titer was 1:2000 to 8000, and the lymphocytotoxicity was 1:4500 to 36000. The loss of ALGG and leuko-agglutination in the total process was about half.

Immunosuppression: The antidog ALGG which had a leuko-agglutination titer of 1:4000 was given for two days before and daily after renal homotransplantation (doses 0.125 ml./kg.). With survival credit of individual dogs limited to 50 days, the 11 animals had a mean survival of 23.2 ± 15.7 (SD) as compared with 13.4 ± 7.8 (SD) days in 10 untreated controls ($p < 0.05$). The results were comparable to those previously reported with ammonium sulphate precipitated ALG but worse than with raw ALS (3).

The antihuman ALGG was tested by Balner (4) in chimpanzees and found to have moderate (but not superior) immunosuppressive qualities. It was given to 14 kidney homograft recipients as an adjuvant agent. The toxicity was less than with the previously used ammonium sulphate precipitated ALG. However, 6 of the 14 patients experienced severe rejection, something rarely observed in the preceding 18 months when less purified globulin (ALG) was being given. Thus, the reduction in side effects may have been at the cost of therapeutic efficacy.

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NEUTROPHIL FUNCTION AND INFECTION DURING IMMUNOSUPPRESSION AND TRANSPLANTATION

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THE ADVERSE EFFECTS of immunosuppressive therapy on nonspecific host defense mechanisms are etiologically important in the genesis of infectious complications in the modified transplant recipient. The influence of steroids and antilymphocyte globulin on the inflammatory response has been well documented, but the effect of these and other agents on the ability of neutrophils to destroy bacteria in transplant recipients has not been studied adequately. Details of the technique for studying neutrophil function are described elsewhere (1, 2).

METHOD

Serial measurements of the ability of isolated neutrophils to phagocytize and kill *Staphylococcus aureus* 502A were performed in 60 female dogs with renal allografts divided into two control groups (unmodified recipients and those treated only with normal goat globulin) and into six treatment groups (modified with goat antilymphocyte globulin, azathioprine, prednisone, and combinations thereof). Serial measurements of neutrophil function were also made in six normal dogs treated only with azathioprine and/or prednisone and in six animals with orthotopic hepatic allografts modified by antilymphocyte globulin, azathioprine, and prednisone. In addition

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